Plants having modified growth characteristics and a method for making the same

The present invention concerns a method for modifying growth characteristics of a plant. More specifically, the present invention concerns a method for modifying growth characteristics of a plant by modifying expression of a seedy1 nucleic acid and/or by modifying levels and/or activity of a seedy1 protein in a plant. The present invention also concerns plants having modified growth characteristics and modified expression of a seedy1 nucleic acid and/or modified levels and/or activity of a seedy1 protein relative to corresponding wild type plants.

The ever-increasing world population and the dwindling supply of arable land available for agriculture fuel research towards improving the efficiency of agriculture. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogeneous genetic components that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to modify the germplasm of animals and plants. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has the capacity to deliver crops or plants having various improved economic, agronomic or horticultural traits. A trait of particular economic interest is yield. Yield is normally defined as the measurable produce of economic value from a crop. This may be defined in terms of quantity and/or quality. Crop yield may not only be increased by combating one or more stresses to which a crop or plant is typically subjected, but may also be increased by modifying the inherent growth characteristics of a plant. Yield is directly dependent on several growth characteristics, for example, growth rate, biomass production, plant architecture, number and size of organs, (for example, the number of branches, tillers, shoots, flowers), seed production and more. Root development and nutrient uptake may also be important factors in determining yield.

The ability to modify one or more plant growth characteristics, would have many applications in areas such as crop enhancement, plant breeding, production of ornamental plants, aboriculture, horticulture, forestry, production of algae or plants (for example for use as bioreactors, for the production of substances such as pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic waste or for use as fuel in the case of high-yielding algae and plants).

It has now been found that modifying expression in a plant of a seedy1 nucleic acid and/or modifying the level and/or activity in a plant of a seedy1 protein gives plants having modified growth characteristics relative to corresponding wild type plants.

A seedy1 protein is defined herein as being a protein comprising in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16; and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17, and which motif is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18.

A seedy1 nucleic acid/gene is defined herein as being a nucleic acid or gene encoding a seedy1 protein. The terms "seedy1 gene", "seedy1 nucleic acid" and "nucleic acid encoding a seedy1 protein" are used interchangeably herein. The term seedy1 nucleic acid/gene, as defined herein, also encompasses a complement of the sequence and corresponding RNA, DNA, cDNA or genomic DNA. The seedy1 nucleic acid may be synthesized in whole or in part and it may be a double-stranded nucleic acid or a single-stranded nucleic acid. The term also encompasses variants due to the degeneracy of the genetic code and variants that are interrupted by one or more intervening sequences.

A seedy1 nucleic acid/gene or a seedy1 protein may be wild type, i.e. a native or endogenous nucleic acid or protein. The nucleic acid may be derived from the same or another species, which nucleic acid is introduced as a transgene, for example by transformation. This transgene may be substantially changed from its native form in composition and/or genomic environment through deliberate human manipulation. The nucleic acid may thus be derived (either directly or indirectly (if subsequently modified)) from any source provided that the nucleic acid, when expressed in a plant, gives modified plant growth characteristics. The nucleic acid may be isolated from a microbial source, such as bacteria, yeast or fungi, or from a plant, algae, insect, or animal (including human) source. Preferably, the seedy1 nucleic acid is isolated from a plant. The nucleic acid may be isolated from a dicotyledonous species, preferably from the family *Solanaceae*, further preferably from *Nicotiana*. More preferably, the seedy1 nucleic acid encodes a seedy1 protein as defined hereinabove. Most preferably, the seedy1 nucleic acid is as represented by SEQ ID NO: 1, or by a portion thereof, or by a nucleic acid capable of

hybridising with the sequence represented by SEQ ID NO: 1, or is a nucleic acid encoding an amino acid represented by SEQ ID NO: 2 or a homologue derivative or active fragment thereof, which homologue has in increasing order of preference at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% 98% or 99% sequence identity with the sequence represented by SEQ ID NO 2.

The present invention provides a method for modifying the growth characteristics of a plant, comprising modifying expression in a plant of a nucleic acid encoding a seedy1 protein and/or modifying the level and/or activity in a plant of a seedy1 protein, wherein said seedy1 protein comprises in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16, and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17 and which is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18,

wherein the growth characteristics are modified relative to the growth characteristics of corresponding wild-type plants.

The present invention also provides a hitherto unknown seedy1 protein, which seedy1 protein comprises in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16; and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17 and which motif is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18,

with the proviso that the seedy1 protein is not the *Arabidopsis* protein as deposited in Genbank under NCBI accession number AL161572 (SEQ ID NO 12).

According to a particular embodiment, the motif according to SEQ ID NO: 15 is as represented by: (P/X)X((V/L/H)(Q/H)(V/I)W(N/X)NA(A/P)(F/C)D, wherein X may be any amino acid and wherein

(P/X) preferably is P or is A or T or Q or another amino acid
(V/L/H) preferably is V or L or H
(Q/H) is either Q or H
(V/I) is either V or is T or S or another amino acid
(A/P) is preferably A or is P
(F/C) is preferably F or is C.

According to a particular embodiment, the motif according to SEQ ID NO 17 is as represented by: (I/V/A)(D/E)XE(I/M)XX(I/V)(E/Q)XE(I/X)XRL(S/X)(S/X)(R/K)LXXLR (L/V/T/I)X(K/Q), where X may be any amino acid and wherein:
(I/V/A) preferably is I or V or is A
(D/E) is either D or E
(I/M) preferably is I or is M
(I/V) preferably is I or is V
(E/Q) preferably is E or is Q
(I/X) preferably is I or is M or is V or any other amino acid
(S/X) preferably S or is T or any other amino acid
(S/X) preferably is S or is T or L or I or A
(R/K) preferably is R or is K
(L/V/T/I) preferably is L or T or V or I
(K/Q) preferably is K or Q
and which motif is a coiled coil motif.

According to a particular embodiment, the motif according to SEQ ID NO 18 is as represented by: LP(R/K)I(R/X)(T/I)(M/X)(P/R)XX(D/X)(E/G)(S/T)(P/L)RDSG(C/X)(A/X)KR(V/X)(A/I)(D/E)(L/R)(V/X)(G/A)K, where X may be any amino acid and wherein
(R/K) is either R or K
(R/X) is preferably R or is S or K
(T/I) is preferably T or I
(M/X) is preferably M or L or A or V
(P/R) is either P or R
(D/X) is preferably D or is G or T or N
(E/G) is preferably E or is G
(S/T) is preferably S or is T
(P/L) is preferably P or is L
(C/X) is preferably C or is P or A
(A/X) is preferably A or is V or I

(A/I) is preferably A or is I
(D/E) is either D or E
(L/R) is preferably L or is R
(V/X) is preferably V or is Q or N or I
(G/A) is preferably G or is A.

The present invention also provides a hitherto unknown isolated seedy1 nucleic acid/gene selected from:

(i) a nucleic acid represented by any one of SEQ ID NO: 1, 5 or 7 or the complement of any of the aforementioned;

(ii) a nucleic acid encoding an amino acid sequence represented by SEQ ID NO: 2, 4, 6, 8 or 10;

(iii) a nucleic acid encoding a homologue, derivative or active fragment of (i) or (ii) above;

(iv) a nucleic acid capable of hybridising with a nucleic acid of (i), (ii) or (iii) above;

(v) a nucleic acid which is degenerate as a result of the genetic code from any one of the nucleic acids of (i) to (iv) above;

(vi) a nucleic acid which is an allelic variant of any one of the nucleic acids of (i) to (v) above;

(vii) a nucleic acid which is an alternative splice variant of any one of the nucleic acids of (i) to (vi);

(viii) a nucleic acid encoding a protein which has in increasing order of preference at least 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one or more from the sequences defined in (i) to (vii) above;

(ix) a portion of a nucleic acid according to any of (i) to (viii) above;

wherein the nucleic acids of (i) to (ix) above encode a seedy1 protein as defined hereinabove, and with the proviso that the isolated nucleic acid is not a rice cDNA as deposited under Genbank accession number AK063941 (SEQ ID NO 3), a *Medicago* BAC clone deposited as AC144618, AC139356, AC144482 or AC135566, the *Arabidopsis* cDNA deposited under AL61572 (SEQ ID NO 11) or the *Zea mays* EST deposited under AY108162 (SEQ ID NO 9).

Modifying expression of a seedy1 nucleic acid and/or modifying activity and/or levels of a seedy1 protein may be effected by modifying expression of a gene and/or by modifying activity and/or levels of a gene product, namely a polypeptide, in specific cells or tissues. The term

"modifying" as used herein (in the context of modifying expression, activity and/or levels) means increasing, decreasing or changing in time or place. The modified expression, activity and/or levels of a seedy1 gene or protein are modified compared to expression, activity and/or levels of a seedy1 gene or protein in corresponding wild-type plants. The modified gene expression may result from modified expression levels of an endogenous seedy1 gene and/or may result from modified expression levels of a seedy1 gene introduced into a plant. Similarly, levels and/or activity of a seedy1 protein may be modified due to modified expression of an endogenous seedy1 nucleic acid/gene and/or due to modified expression of a seedy1 nucleic acid/gene introduced into a plant. Activity of a seedy1 protein may be increased by increasing levels of the protein itself. Activity may also be increased without any increase in levels of a seedy1 protein or even when there is a reduction in levels of a seedy1 protein. This may occur when the intrinsic properties of the polypeptide are altered, for example, by making a mutant form that is more active than the wild type. Mutations may cause conformational changes in a protein, resulting in more activity and/or levels of a protein. Modified expression of a gene/nucleic acid and/or modifying activity and/or levels of a gene product/protein may be effected, for example, by introducing a genetic modification (preferably in the locus of a seedy1 gene). The locus of a gene as defined herein is taken to mean a genomic region which includes the gene of interest and 10KB up- or down stream of the coding region.

The genetic modification may be introduced, for example, by any one (or more) of the following methods: TDNA activation, tilling, site-directed mutagenesis, homologous recombination or by introducing and expressing in a plant a nucleic acid encoding a seedy1 protein or a homologue, derivative or active fragment thereof. Following introduction of the genetic modification, there follows a step of selecting for increased expression and/or activity and/or levels of a seedy1 protein, which increase in expression and/or activity and/or levels gives plants having modified growth characteristics.

T-DNA activation tagging (Hayashi *et al.* Science (1992) 1350-1353) involves insertion of T-DNA usually containing a promoter (may also be a translation enhancer or an intron), in the genomic region of the gene of interest or 10KB up- or downstream of the coding region of a gene in a configuration such that the promoter directs expression of the targeted gene. Typically, regulation of expression of the targeted gene by its natural promoter is disrupted and the gene falls under the control of the newly introduced promoter. The promoter is typically embedded in a T-DNA. This T-DNA is randomly inserted into the plant genome, for example, through *Agrobacterium* infection and leads to overexpression of genes near to the inserted T-DNA. The resulting transgenic plants show dominant phenotypes due to overexpression of genes close to the introduced promoter. The promoter to be introduced may be any promoter

capable of directing expression of a gene in the desired organism, in this case a plant. For example, constitutive, tissue-preferred, cell type-preferred and inducible promoters are all suitable for use in T-DNA activation.

A genetic modification may also be introduced in the locus of a seedy1 gene using the technique of TILLING (Targeted Induced Local Lesions IN Genomes). This is a mutagenesis technology useful to generate and/or identify, and to isolate mutagenised variants of a seedy1 nucleic acid. TILLING also allows selection of plants carrying such mutant variants. These mutant variants may even exhibit higher seedy1 activity than exhibited by the gene in its natural form. TILLING combines high-density mutagenesis with high-throughput screening methods. The steps typically followed in TILLING are: (a) EMS mutagenesis (Redei and Koncz, 1992; Feldmann *et al.*, 1994; Lightner and Caspar, 1998); (b) DNA preparation and pooling of individuals; (c) PCR amplification of a region of interest; (d) denaturation and annealing to allow formation of heteroduplexes; (e) DHPLC, where the presence of a heteroduplex in a pool is detected as an extra peak in the chromatogram; (f) identification of the mutant individual; and (g) sequencing of the mutant PCR product. Methods for TILLING are well known in the art (McCallum Nat Biotechnol. 2000 Apr; 18(4): 455-7, reviewed by Stemple 2004 (TILLING--a high-throughput harvest for functional genomics. Nat Rev Genet. 2004 Feb; 5(2):145-50.)).

Site directed mutagenesis may be used to generate variants of seedy1 nucleic acids or portions thereof. Several methods are available to achieve site directed mutagenesis; the most common being PCR based methods (current protocols in molecular biology. Wiley Eds. http://www.4ulr.com/products/currentprotocols/index.html).

TDNA activation, TILLING and site-directed mutagenesis are examples of technologies that enable the generation of novel alleles and seedy1 nucleic acid variants that are therefore useful in the methods of the invention.

Homologous recombination allows introduction in a genome of a selected nucleic acid at a defined selected position. Homologous recombination is a standard technology used routinely in the biological sciences for lower organisms such as yeast or moss (e.g. physcomitrella). Methods for performing homologous recombination in plants have been described not only for model plants (Offringa *et al.* Extrachromosomal homologous recombination and gene targeting in plant cells after *Agrobacterium*-mediated transformation, 1990 EMBO J. 1990 Oct; 9(10):3077-84) but also for crop plants, for example rice (Terada R, Urawa H, Inagaki Y, Tsugane K, Iida S. Efficient gene targeting by homologous recombination in rice. Nat

Biotechnol. 2002. Iida and Terada: A tale of two integrations, transgene and T-DNA: gene targeting by homologous recombination in rice. Curr Opin Biotechnol. 2004 Apr; 15(2):132-8). The nucleic acid to be targeted need not be targeted to the locus of a seedy1 gene, but may be introduced in, for example, regions of high expression. The nucleic acid to be targeted may be an improved allele used to replace the endogenous gene or may be introduced in addition to the endogenous gene.

A preferred method for introducing a genetic modification is to introduce and express in a plant a seedy1 nucleic acid/gene or a portion thereof, or sequences capable of hybridising with the seedy1 nucleic acid/gene, which nucleic acid encodes a seedy1 protein or a homologue, derivative or active fragment thereof. In this case, the genetic modification need not be in the locus of a seedy1 gene. The nucleic acid may be introduced into a plant by, for example, transformation.

Accordingly, the present invention provides a method for modifying the growth characteristics of a plant, comprising introducing and expressing in a plant a seedy1 nucleic acid/gene or a portion thereof, or sequences capable of hybridising with the seedy1 nucleic acid/gene, which nucleic acid encodes a seedy1 protein or a homologue, derivative or active fragment thereof.

Advantageously, the methods according to the invention may also be practised using variant nucleic acids and variant amino acids of SEQ ID NO 1 or 2 respectively. The term seedy1 nucleic acid or seedy1 protein encompasses variant nucleic acids and variant amino acids. The variant nucleic acids encode seedy1 proteins as defined hereinabove, i.e. those comprising in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16; and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17, and which motif is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18,

and variant seedy1 proteins are those comprising in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16; and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17, and which motif is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18.

Suitable variant nucleic acid and amino acid sequences useful in practising the method according to the invention, include:

(i) Portions of a seedy1 nucleic acid/gene;

(ii) Sequences capable of hybridising with a seedy1 nucleic acid/gene;

(iii) Alternative splice variants of a seedy1 nucleic acid/gene;

(iv) Allelic variants of a seedy1 nucleic acid/gene;

(v) Homologues, derivatives and active fragments of a seedy1 protein.

An example of a variant seedy1 nucleic acid is a portion of a seedy1 nucleic acid. The methods according to the invention may advantageously be practised using functional portions of a seedy1 nucleic acid. A portion refers to a piece of DNA derived or prepared from an original (larger) DNA molecule, which DNA portion, when introduced and expressed in a plant, gives plants having modified growth characteristics and which portion encodes a seedy1 protein as defined hereinabove. The portion may comprise many genes, with or without additional control elements or may contain spacer sequences. The portion may be made by making one or more deletions and/or truncations to the nucleic acid. Techniques for introducing truncations and deletions into a nucleic acid are well known in the art. Portions suitable for use in the methods according to the invention may readily be determined by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the portion to be tested for functionality.

An example of a further variant seedy1 nucleic acid is a sequence that is capable of hybridising to a seedy1 nucleic acid as defined hereinabove, for example to a seedy1 nucleic acid as represented by any one of SEQ ID NO 1, 3, 5, 7, 9 or 11. Such hybridising sequences are those encoding a seedy1 protein as defined hereinabove. Hybridising sequences suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the hybridising sequence.

The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridisation, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to e.g. a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridisation, plaque hybridisation, *in situ* hybridisation and microarray hybridisation. In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration and hybridisation buffer composition. High stringency conditions for hybridisation include high temperature and/or low salt concentration (salts include NaCl and Na_3-citrate) and/or the inclusion of formamide in the hybridisation buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridisation buffer and/or exclusion of compounds such as dextran sulphate or polyethylene glycol (promoting molecular crowding) from the hybridisation buffer. Conventional hybridisation conditions are described in, for example, Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York, but the skilled craftsman will appreciate that numerous different hybridisation conditions can be designed in function of the known or the expected homology and/or length of the nucleic acid. Sufficiently low stringency hybridisation conditions are particularly preferred (at least in the first instance) to isolate nucleic acids heterologous to the DNA sequences of the invention defined supra. An example of low stringency conditions is 4-6x SSC / 0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of the nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed, such as medium stringency conditions. Examples of medium stringency conditions include 1-4x SSC / 0.25% w/v SDS at ≥ 45°C for 2-3 hours. An example of high stringency conditions includes 0.1-1x SSC / 0.1% w/v SDS at 60°C for 1-3 hours. The skilled man will be aware of various parameters which may be altered during hybridisation and washing and which will either

maintain or change the stringency conditions. The stringency conditions may start low and be progressively increased until there is provided a hybridising seedy1 nucleic acid, as defined hereinabove. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage.

Another example of a variant seedy1 is an alternative splice variant of a seedy1 nucleic acid/gene. The methods according to the present invention may also be practised using an alternative splice variant of a seedy1 nucleic acid. The term "alternative splice variant" as used herein encompasses variants of a nucleic acid in which selected introns and/or exons have been excised, replaced or added. Such splice variants may be found in nature or can be manmade using techniques well known in the art. Preferably, the splice variant is a splice variant of a sequence represented by any of SEQ ID NO 1, 3, 5, 7, 9 or 11. Splice variants suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the splice variant.

Another example of a variant seedy1 is an allelic variant. Advantageously, the methods according to the present invention may also be practised using allelic variants of a seedy1 nucleic acid, preferably an allelic variant of a seedy1 nucleic acid sequence represented by any of SEQ ID NO 1, 3, 5, 7, 9 or 11. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these isolated natural alleles in the methods according to the invention. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp). SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms. Allelic variants suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the allelic variant.

Examples of variant seedy1 amino acids include homologues, derivatives and active fragments of a seedy1 protein, preferably of a seedy1 protein as represented by any one of SEQ ID NO 2, 4, 6, 8, 10 or 12. Homologues, derivatives and active fragments of a seedy1 protein are those comprising in the following order from N-terminus to C-terminus:

(i) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 15; and

(ii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 16; and

(iii) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 17, and which motif is a coiled coil motif; and

(iv) a motif having at least 80% sequence identity to the sequence represented by SEQ ID NO 18.

Preferred seedy1 homologues, derivatives and active fragments have a coiled coil domain, preferably located in the N-terminal half of the protein, more preferably between amino acid position 25 to 250, more preferably between position 50 and 150. A coiled coil domain typically determines protein folding.

"Homologues" of a seedy1 protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unchanged protein in question and having similar biological and functional activity as the unchanged protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company).

The homologues of a seedy1 protein have a percentage identity to any one of SEQ ID NO 2, 4, 6, 8, 10 or 12 equal to a value lying between 20% and 99.99%, i.e. in increasing order of preference at least 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, or 50% sequence identity or similarity (functional identity) to the unchanged protein, alternatively at least 60% sequence identity or similarity to an unchanged protein, alternatively at least 70% sequence identity or similarity to an unchanged protein. Typically, the homologues have at least 75% or 80% sequence identity or similarity to an unchanged protein, preferably at least 85%, 86%, 87%, 88%, 89% sequence identity or similarity, further preferably at least 90%, 91%, 92%, 93%, 94% sequence identity or similarity to an unchanged protein, most preferably at least 95%, 96%, 97%, 98% or 99% sequence identity or similarity to an unchanged protein. The percentage identities are when comparing full-length sequences. Homologues suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the homologous sequence.

Percentage identity may be calculated using an alignment program, such alignment programs being well known in the art. For example, percentage identity may be calculated using the

program GAP, or needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 5.5 software package, using the standard parameters (for example GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

Methods for the search and identification of seedy1 homologues or DNA sequences encoding a seedy1 homologue, would be well within the realm of persons skilled in the art. Such methods, involve screening sequence databases with the sequences as provided by the present invention in SEQ ID NO 1 and 2 or 3 to 10, preferably a computer readable format of the nucleic acids of the present invention. This sequence information is available for example in public databases, that include but are not limited to Genbank (http://www.ncbi.nlm.nih.gov/web/Genbank), the European Molecular Biology Laboratory Nucleic acid Database (EMBL) (http:/w.ebi.ac.uk/ebi-docs/embl-db.html) or versions thereof or the MIPS database (http://mips.gsf.de/). Different search algorithms and software for the alignment and comparison of sequences are well known in the art. Such software includes GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percentage sequence identity and performs a statistical analysis of the similarity between the two sequences. The suite of programs referred to as BLAST programs has 5 different implementations: three designed for nucleotide sequence queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology: 76-80, 1994; Birren et al., GenomeAnalysis, 1: 543, 1997). The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information.

Homologues of SEQ ID NO 2 may be found in many different organisms. The closest homologues are found in the plant kingdom. For example, seedy1 proteins were isolated from tobacco (SEQ ID NO 2), rice (SEQ ID NO 4), medicago (SEQ ID NO 6), sugar cane (SEQ ID NO 8), maize (SEQ ID NO 10) and from *Arabidopsis* (SEQ ID NO 12). Furthermore, ESTs from other organisms have been deposited in Genbank, for example an EST from *Vitis vinifera* (accession number CA816066), from *Pinus taeda* (accession number BM903108), from *Saccharus sp.* (accession numbers CA228193 and CA256020), from *Citrus sinsensis* (accession number CF833583), *Plumbago zeylanica* (accession number CB817788), from *Zea mays* (accession number CF637447, AW282224, CD058812, AY108162, CD059048, CF041861, AW067243), from *Triticum aestivum* (CA727065, BJ264506, BJ259034), from *Hordeum vulgare* (accession number BU997034, CA727065, CA031127, BQ762011), from

Brassica napus (CD817460) from *Gossypium arboreum* (BG446106, BM360339), from *Eschscholzia californica* (CD478368), from *Populus tremula* (BU821376) and from *Beta vulgaris* (BQ594009). As more genomes are sequenced, many more seedy1 homologues will be identified.

The identification of domains or motifs, would also be well within the realm of a person skilled in the art and involves for example, a computer readable format of the nucleic acids of the present invention, the use of alignment software programs and the use of publicly available information on protein domains, conserved motifs and boxes. This protein domain information is available in the PRODOM (http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html), PIR (http://pir.georgetown.edu/) or pFAM (http://pfam.wustl.edu/) database. Sequence analysis programs designed for motif searching may be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 3.0) can be found in the GCG package; or on the Internet site http://www.sdsc.edu/MEME/meme. SIGNALSCAN version 4.0 information is available on the Internet site http://biosci.cbs.umn.edu/software/sigscan.html. GENESCAN can be found on the Internet site http://gnomic.stanford.edu/GENESCANW.html.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogous" relates to gene-duplications within the genome of a species. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship and the formation of different species. The term "homologue" as defined herein also encompasses paralogues and orthologues.

Othologues in, for example, monocot plant species may easily be found by performing a so-called reciprocal blast search. This may be done by a first blast involving blasting the sequence in question (for example, SEQ ID NO: 1 or SEQ ID NO: 2) against any sequence database, such as the publicly available NCBI database which may be found at: http://www.ncbi.nlm.nih.gov. If orthologues in rice were sought, the sequence in question would be blasted against, for example, the 28,469 full-length cDNA clones from *Oryza sativa* Nipponbare available at NCBI. BLASTn or tBLASTX may be used when starting from nucleotides or BLASTP or TBLASTN when starting from the protein, with standard default values. The blast results may be filtered. The full-length sequences of either the filtered results or the non-filtered results are then blasted back (second blast) against the sequences

of the organism from which the sequence in question is derived. The results of the first and second blasts are then compared. An orthologue is found when the results of the second blast give as hits with the highest similarity a seedy1 nucleic acid or protein; if one of the organisms is tobacco then a paralogue is found. In the case of large families, ClustalW may be used, followed by a neighbour joining tree, to help visualize the clustering.

Example homologues of a seedy1 protein according to SEQ ID NO: 2 include a seedy1 protein as represented by SEQ ID NO 4 (rice), SEQ ID NO 8 (sugar cane) and SEQ ID NO 10 (maize), SEQ ID NO 6 (medicago) and SEQ ID NO 12 (*Arabidopsis*). The proteins represented by SEQ ID NO 8 (sugar cane) and SEQ ID NO 10 (Maize) are only partial, but the corresponding full length sequences of the proteins and encoding cDNA may easily be determined by a person skilled in the art using routine techniques, such as colony hybridization of a cDNA library or using PCR based on the use of specific primers combined with degenerate primers.

Another variant of seedy1 useful in the methods of the present invention is a derivative of seedy1. The term "derivatives" refers to peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise substitutions, deletions or additions of naturally and non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the protein, for example, as presented in SEQ ID NO: 2. "Derivatives" of a seedy1 protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring changed, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein.

"Substitutional variants" of a protein are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1 to 10 amino acid residues, and deletions will range from about 1 to 20 residues. Preferably, amino acid substitutions comprise conservative amino acid substitutions.

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in a protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)6-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag·100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein are characterised by the removal of one or more amino acids from the protein. Amino acid variants of a protein may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen in vitro mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

Another variant of a seedy1 protein/amino acid useful in the methods of the present invention is an active fragment of a seedy1 protein. "Active fragments" of a seedy1 protein encompass contiguous amino acid residues of a seedy1 protein, which residues retain similar biological and/or functional activity to the naturally occurring protein. Useful fragments are those falling within the definition of a seedy1 protein as defined hereinabove. Preferably, the fragments start at one of the second or third or further internal methionine residues. These fragments originate from protein translation, starting at internal ATG codons.

For determining the presence of conserved motifs, sequences are aligned using suitable software, such as Align X or clustal X, for indication of the conserved residues (see for example Figure 3). Software packages like MEME version 3.0 may also be used to determine motifs in sequences. This software is available from UCSD, SDSC and NBCR at http://meme.sdsc.edu/meme/. For the identification of a coiled coil domain, the software Coils 2.0 can be used. This software is available at http://www.ch.embnet.org/software/COILS_form.html. The 'X' in the motifs represented by SEQ ID NO 15, 16, 17 and 18 represents any amino acid.

According to a preferred aspect of the present invention, enhanced or increased expression of a seedy1 nucleic acid in a plant or plant part is envisaged. Methods for obtaining increased expression of genes or gene products are well documented in the art and include, for example, overexpression driven by a (strong) promoter, the use of transcription enhancers or translation enhancers. The term overexpression as used herein means any form of expression that is additional to the original wild-type expression level. Preferably the seedy1 nucleic acid is in the sense direction with respect to the promoter to which it is operably linked. Alternatively, selection of better performing alleles of the wild-type seedy1 nucleic acid can be achieved via plant breeding techniques.

The expression of a seedy1 gene may be investigated by Northern or Southern blot analysis of cell extracts. The levels of a seedy1 protein in cells may be investigated using Western blot analysis of cell extracts.

According to a further embodiment of the present invention, genetic constructs and vectors to facilitate introduction and/or expression of the nucleotide sequences useful in the methods according to the invention are provided. Therefore, the present invention provides a genetic construct comprising:

(i) A seedy1 nucleic acid encoding a seedy1 protein as defined hereinabove;

(ii) one or more control sequences capable of regulating expression of the nucleic acid of (i); and optionally

(iii) a transcription termination sequence.

According to methods of the present invention, such a genetic construct is introduced into a plant or plant part.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.

The genetic construct may be an expression vector wherein said nucleic acid is operably linked to one or more control sequences allowing expression in prokaryotic and/or eukaryotic host cells.

The nucleic acid according to (i) may be any seedy1 nucleic acid as defined hereinabove, preferably a seedy1 nucleic acid as represented by any one of SEQ ID NO 1, 3, 5, 7, 9 or 11. The control sequence of (ii) is preferably a seed-preferred promoter, for example a prolamin promoter.

Plants are transformed with a vector comprising the sequence of interest, which sequence is operably linked to one or more control sequences (at least a promoter). The terms "regulatory element", "control sequence" are all used interchangeably herein and are to be taken in a broad context to refer to regulatory nucleic acids capable of effecting expression of the sequences to which they are ligated (i.e. operably linked). Encompassed by the aforementioned terms are promoters. A "Promoter" encompasses transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a –35 box sequence and/or –10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Advantageously, any type of promoter may be used to drive expression of the seedy1 nucleic acid. Preferably, the nucleic acid capable of modifying expression of a seedy1 gene is operably linked to a plant-derived promoter, preferably a plant-derived tissue-preferred promoter. The term "tissue-preferred" as defined herein refers to a promoter that is expressed predominantly in at least one tissue or organ. Preferably, the tissue-preferred promoter is a seed-preferred promoter or a seed-specific promoter, further preferably an endosperm-preferred promoter, more preferably a promoter isolated from a gene encoding a seed-storage protein, most preferably a promoter isolated from a prolamin gene, such as a rice prolamin promoter as represented by SEQ ID NO 14 or a promoter of similar strength and/or a promoter with a similar expression pattern as the rice prolamin promoter. Similar strength and/or similar expression pattern may be analysed, for example, by coupling the promoters to a reporter gene and checking the function of the reporter gene in tissues of the plant. One well-known

reporter gene is beta-glucuronidase and the colorimetric GUS stain used to visualize beta-glucuronidase activity in plant tissue.

Examples of preferred seed-specific promoters and other tissue-specific promoters are presented in Table A, which promoters or derivatives thereof are useful in performing the methods of the present invention.

TABLE A

EXAMPLES OF SEED-PREFERRED PROMOTERS FOR USE IN THE PRESENT INVENTION		
GENE SOURCE	**EXPRESSION PATTERN**	**REFERENCE**
seed-specific genes	seed	Simon, *et al., Plant Mol. Biol. 5:* 191, 1985; Scofield, *et al., J. Biol. Chem. 262:* 12202, 1987.; Baszczynski, *et al., Plant Mol. Biol. 14:* 633, 1990.
Brazil Nut albumin	seed	Pearson, et al., Plant Mol. Biol. 18: 235-245, 1992.
legumin	seed	Ellis, et al., Plant Mol. Biol. 10: 203-214, 1988.
glutelin (rice)	seed	Takaiwa, et al., Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, et al., FEBS Letts. 221: 43-47, 1987.
zein	seed	Matzke et al Plant Mol Biol, 14(3):323-32 1990
napA	seed	Stalberg, *et al, Planta 199:* 515 -519, 1996.
wheat LMW and HMW glutenin-1	endosperm	Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989
wheat SPA	seed	Albani *et al,* Plant Cell, 9: 171-184, 1997
wheat α, β, γ-gliadins	endosperm	EMBO 3:1409-15, 1984
barley *ltr1* promoter	endosperm	
barley B1, C, D, hordein	endosperm	Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996
barley DOF	endosperm	Mena *et al,* The Plant Journal, 116(1): 53-62, 1998
blz2	endosperm	EP99106056.7
synthetic promoter	endosperm	Vicente-Carbajosa *et al., Plant J. 13:*

		629-640, 1998.
rice prolamin NRP33	endosperm	Wu *et al*, Plant Cell Physiology 39(8) 885-889, 1998
rice α-globulin Glb-1	endosperm	Wu *et al*, Plant Cell Physiology 39(8) 885-889, 1998
rice OSH1	embryo	Sato *et al*, Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996
rice α-globulin REB/OHP-1	endosperm	Nakase *et al.* Plant Mol. Biol. 33: 513-522, 1997
rice ADP-glucose PP	endosperm	Trans Res 6:157-68, 1997
maize ESR gene family	endosperm	Plant J 12:235-46, 1997
sorgum γ-kafirin	endosperm	PMB 32:1029-35, 1996
KNOX	embryo	Postma-Haarsma *et al*, Plant Mol. Biol. 39:257-71, 1999
rice oleosin	embryo and aleuron	Wu *et at*, J. Biochem., 123:386, 1998
sunflower oleosin	seed (embryo and dry seed)	Cummins, *et al.*, Plant Mol. Biol. 19: 873-876, 1992
Metallothionein Mte, PRO0001		transfer layer of embryo + calli
putative beta-amylase, PRO0005		transfer layer of embryo
putative cellulose synthase, PRO0009		weak in roots
lipase (putative), PRO0012		
transferase (putative), PRO0014		
peptidyl prolyl cis-trans isomerase (putative), PRO0016		
Unknown, PRO0019		
prp protein (putative), PRO0020		
noduline (putative), PRO0029		
proteinase inhibitor Rgpi9, PRO0058		seed
beta expansine EXPB9, PRO0061		weak in young flowers
structural protein, PRO0063		young tissues+calli+embryo
xylosidase (putative), PRO0069		
prolamine 10 Kda, PRO0075		strong in endosperm
allergen RA2, PRO0076		strong in endosperm
prolamine RP7, PRO0077		strong in endosperm
CBP80, PRO0078		
starch branching enzyme I, PRO0079		
Metallothioneine-like ML2, PRO0080		transfer layer of embryo + calli
putative caffeoyl-CoA 3-O-methyltransferase, PRO0081		shoot
prolamine RM9, PRO0087		strong in endosperm
prolamine RP6, PRO0090		strong endosperm

prolamine RP5, PRO0091	strong in endosperm
allergen RA5, PRO0092	
putative methionine aminopeptidase, PRO0095	embryo
ras-related GTP binding protein, PRO0098	
beta expansine EXPB1, PRO0104	
Glycine rich protein, PRO0105	
metallothionein like protein (putative), PRO0108	
metallothioneine (putative), PRO0109	
RCc3, PRO0110	strong root
uclacyanin 3-like protein, PRO0111	weak discrimination center / shoot meristem
26S proteasome regulatory particle non-ATPase subunit 11, PRO0116	very weak meristem specific
putative 40S ribosomal protein, PRO0117	weak in endosperm
chlorophyll a/b-binding protein presursor (Cab27), PRO0122	very weak in shoot
putative protochlorophyllide reductase, PRO0123	strong leaves
metallothionein RiCMT, PRO0126	strong discrimination center / shoot meristem
GOS2, PRO0129	strong constitutive
GOS9, PRO0131	
chitinase Cht-3, PRO0133	very weak meristem specific
alpha-globulin, PRO0135	strong in endosperm
alanine aminotransferase, PRO0136	weak in endosperm
cyclin A2, PRO0138	
Cyclin D2, PRO0139	
Cyclin D3, PRO0140	
cyclophyllin 2, PRO0141	shoot and seed
sucrose synthase SS1 (barley), PRO0146	medium constitutive
trypsin inhibitor ITR1 (barley), PRO0147	weak in endosperm
ubiquitine 2 with intron, PRO0149	strong constitutive
WSI18, PRO0151	embryo + stress
HVA22 homologue (putative), PRO0156	
EL2, PRO0157	
Aquaporine, PRO0169	medium constitutive in young plants
High mobility group protein, PRO0170	strong constitutive
reversibly glycosylated protein RGP1, PRO0171	weak constitutive
cytosolic MDH, PRO0173	shoot
RAB21, PRO0175	embryo + stress
CDPK7, PRO0176	

Cdc2-1, PRO0177	very weak in meristem
sucrose synthase 3, PRO0197	
OsVP1, PRO0198	
OSH1, PRO0200	very weak in young plant meristem
putative chlorophyllase, PRO0208	
OsNRT1, PRO0210	
EXP3, PRO0211	
phosphate transporter OjPT1, PRO0216	
oleosin 18kd, PRO0218	aleurone + embryo
ubiquitine 2 without intron, PRO0219	
RFL, PRO0220	
maize UBI delta intron, PRO0221	
glutelin-1, PRO0223	
fragment of prolamin RP6 promoter, PRO0224	
4xABRE, PRO0225	
glutelin OSGLUA3, PRO0226	
BLZ-2_short (barley), PRO0227	
BLZ-2_long (barley), PRO0228	

Optionally, one or more terminator sequences may also be used in the construct introduced into a plant. The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences, which may be suitable for use in performing the invention. Such sequences would be known or may readily be obtained by a person skilled in the art.

The genetic constructs of the invention may further include an origin of replication sequence which is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

The genetic construct may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a nucleic acid construct of the invention. Suitable markers may be selected

from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as *npt*II that phosphorylates neomycin and kanamycin, or *hpt*, phosphorylating hygromycin), to herbicides (for example *bar* which provides resistance to Basta; *aroA* or *gox* providing resistance against glyphosate), or genes that provide a metabolic trait (such as *manA* that allows plants to use mannose as sole carbon source). Visual marker genes result in the formation of colour (for example β-glucuronidase, GUS), luminescence (such as luciferase) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof).

In a preferred embodiment, the genetic construct comprises a prolamin promoter from rice operably linked to a seedy1 nucleic acid in the sense orientation. An example of such an expression cassette, further comprising a terminator sequence, is as represented by SEQ ID NO 13.

According to a further embodiment of the present invention, there is provided a method for the production of a plant having modified growth characteristics, comprising modifying expression and or activity and/or levels in a plant of a seedy1 nucleic acid or seedy1 protein.

According to a particular embodiment, the present invention provides a method for the production of a transgenic plant having modified growth characteristics, which method comprises:

(i) introducing into a plant or plant part a seedy1 nucleic acid encoding a seedy1 protein;

(ii) cultivating the plant cell under conditions promoting regeneration and mature plant growth.

The nucleic acid of (i) may advantageously be any of the aforementioned seedy1 nucleic acids.

The protein itself and/or the nucleic acid itself may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of the plant). According to a preferred feature of the present invention, the nucleic acid is preferably introduced into a plant by transformation.

The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may

be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g. cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively and preferably, the transgene may be stably integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., 1882, Nature 296, 72-74; Negrutiu I. et al., June 1987, Plant Mol. Biol. 8, 363-373); electroporation of protoplasts (Shillito R.D. et al., 1985 Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A. et al., 1986, Mol. Gen Genet 202, 179-185); DNA or RNA-coated particle bombardment (Klein T.M. et al., 1987, Nature 327, 70) infection with (non-integrative) viruses and the like.

Transgenic rice plants expressing a seedy1 gene are preferably produced via *Agrobacterium*-mediated transformation using any of the well known methods for rice transformation, such as described in any of the following: published European patent application EP 1198985 A1, Aldemita and Hodges (Planta, 199, 612-617, 1996); Chan *et al.* (Plant Mol. Biol. 22 (3) 491-506, 1993), Hiei *et al.* (Plant J. 6 (2) 271-282, 1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida *et al.* (Nat. Biotechnol. 1996 Jun; 14(6): 745-50) or Frame *et al.* (Plant Physiol. 2002 May; 129(1): 13-22), which disclosures are incorporated by reference herein as if fully set forth.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The present invention also encompasses plants obtainable by the methods according to the present invention. The present invention therefore provides plants obtainable by the method according to the present invention, which plants have modified growth characteristics, when compared to wild-type plants.

The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced in the parent by the methods according to the invention i.e. having modified growth characteristics.

The invention accordingly also includes host cells comprising an isolated seedy1 nucleic acid as defined hereinabove. Preferred host cells according to the invention are plant cells or cells from insects, animals, yeast, fungi, algae or bacteria. The invention also extends to harvestable parts of a plant, such as but not limited to seeds, flowers, stamen, leaves, petals, fruits, stem, stem cultures, rhizomes, roots, tubers and bulbs.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells,

tissues and organs. The term "plant" also therefore encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays,* amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice,

soybean, strawberry, sugar beet, sugar cane, sunflower, tomato, squash, tea, trees. Alternatively algae and other non-Viridiplantae can be used for the methods of the present invention. According to a preferred embodiment of the present invention, the plant is a crop plant such as soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato or tobacco. Further preferably, the plant is a monocotyledonous plant, such as sugar cane. More preferably the plant is a cereal, such as rice, maize, wheat, barley, millet, rye, sorghum or oats.

Advantageously, the present invention provides a method for modifying growth characteristics of a plant, which modified growth characteristics are selected from any one or more of increased yield, increased biomass, modified plant architecture.

Further preferably, increased yield is increased seed yield.

The term "increased yield" encompasses an increase in biomass in one or more harvestable parts of a plant relative to the total biomass of corresponding wild-type plants. The term also encompasses an increase in seed yield, which includes an increase in the biomass of the seed (seed weight) and/or an increase in the number of (filled) seeds and/or in the size of the seeds and/or an increase in seed volume, each relative to corresponding wild-type plants. An increase in seed size and/or volume may also influence the composition of seeds. An increase in seed yield could be due to an increase in the number and/or size of flowers. An increase in yield might also increase the harvest index, which is expressed as a ratio of the total biomass over the yield of harvestable parts, such as seeds.

The methods of the present invention are used to increase the seed yield of the plant and are therefore particularly favourable to be applied to crop plants, preferably seed crops and cereals. Therefore, the methods of the present invention are particularly useful for plants such as, rapeseed, sunflower, leguminosae (e.g. soybean, pea, bean) flax, lupinus, canola and cereals such as rice, maize, wheat, barley, millet, oats and rye.

Further preferably, increased biomass encompasses increased biomass of aboveground plant tissue, herein determined as aboveground plant area.
Additionally or alternatively, the plants according to the invention have increased aboveground area relative to corresponding wild type plants.

Further preferably, said modified plant architecture encompasses increased number of panicles and increased biomass relative to corresponding wild type plants.

The present invention also relates to use of a seedy1 nucleic acid and and/or protein in modifying plant growth characteristics.

According to another aspect of the present invention, the seedy1 nucleic acid and/or seedy1 protein may be used in breeding programmes. In an example of such a breeding programme, a DNA marker is identified which may be genetically linked to a seedy1 nucleic acid/gene. This DNA marker may then be used in breeding programs to select plants having modified growth characteristics relative to corresponding wild type plants.

The methods according to the present invention result in plants having modified growth characteristics, as described hereinbefore. These advantageous characteristics may also be combined with other economically advantageous traits, such as further yield-enhancing traits, tolerance to various stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

Description of the Figures

The present invention will now be described with reference to the following figures in which:

Figure 1 is a schematic presentation of the entry clone, containing CDS0689 within the AttL1 and AttL2 sites for Gateway® cloning in the pDONR201 backbone. CDS0689 is the internal code for the *Nicotiana tabacum* BY2 CDS0689 seedy1 coding sequence. This vector contains also a bacterial kanamycine-resistance cassette and a bacterial origin of replication.

Figure 2 is a map of the binary vector for the expression in *Oryza sativa* of the *Nicotiana tabacum* BY2 seedy1 gene (CDS0689) under the control of the rice prolamin RP6 promoter (PRO0090). This vector contains a T-DNA derived from the Ti Plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)). From the left border to the right border, this T-DNA contains: a selectable marker cassette for antibiotic selection of transformed plants; a screenable marker cassette for visual screening of transformed plants; the PRO0090 - CDS0689 -zein and rbcS-deltaGA double terminator cassette for expression of the *Nicotiana tabacum* BY2 seedy1 gene (CDS0689). This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

Figure 3 shows an N-terminal and C-terminal alignment of seedy1 amino acids and deduced amino acids from ESTs, all from plants. This alignment was made with the program Align X of the VNTI software package. Motifs 1, 2, 3 and 4 are indicated with a bar.

Figure 4 is the representation of nucleic acids, protein and motif sequences according to the invention.

Examples

The present invention will now be described with reference to the following examples, which are by way of illustration alone.

Unless otherwise stated, recombinant DNA techniques were performed according to standard protocols described in Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York; or in Volumes 1 and 2 of Ausubel *et al.* (1988), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

Example 1: cloning of the seedy1 encoding gene

A cDNA-AFLP experiment was performed on a synchronized tobacco BY2 cell culture (*Nicotiana tabacum* L. cv. Bright Yellow-2), and BY2 expressed sequence tags that were cell cycle modulated were identified and elected for further cloning. Subsequently, the expressed sequence tags were used to screen a tobacco cDNA library and to isolate the full-length cDNA of interest, namely the cDNA coding for the seedy1 protein of the present invention (CDS0689).

Synchronization of BY2 cells.

Tobacco BY2 (*Nicotiana tabacum* L. cv. Bright Yellow - 2) cultured cell suspension was synchronized by blocking cells in early S-phase with aphidicolin as follows. Cultured cell suspension of *Nicotiana tabacum* L. cv. Bright Yellow 2 were maintained as described (Nagata et al. Int. Rev. Cytol. 132, 1-30, 1992). For synchronization, a 7-day-old stationary culture was diluted 10-fold in fresh medium supplemented with aphidicolin (Sigma-Aldrich, St. Louis, MO; 5 mg/l), a DNA-polymerase a inhibiting drug. After 24 h, cells were released from the block by several washings with fresh medium and resumed their cell cycle progression.

RNA extraction and cDNA synthesis.

Total RNA was prepared by using LiCl precipitation (Sambrook et al, 2001) and poly(A+) RNA was extracted from 500 mg of total RNA using Oligotex columns (Qiagen, Hilden, Germany)

according to the manufacturer's instructions. Starting from 1 mg of poly(A+) RNA, first-strand cDNA was synthesized by reverse transcription with a biotinylated oligo-dT25 primer (Genset, Paris, France) and Superscript II (Life Technologies, Gaithersburg, MD). Second-strand synthesis was done by strand displacement with *Escherichia coli* ligase (Life Technologies), and DNA polymerase I (USB, Cleveland, OH) and RNAse-H (USB).

cDNA-AFLP analysis.

Five hundred ng of double-stranded cDNA was used for AFLP analysis as described (Vos et al., Nucleic Acids Res. 23 (21) 4407-4414, 1995; Bachem et al., Plant J. 9 (5) 745-53, 1996). The restriction enzymes used were BstYI and MseI (Biolabs) and the digestion was done in two separate steps. After the first restriction digest with one of the enzymes, the 3' end fragments were collected on Dyna beads (Dynal, Oslo, Norway) by means of their biotinylated tail, while the other fragments were washed away. After digestion with the second enzyme, the released restriction fragments were collected and used as templates in the subsequent AFLP steps. For preamplifications, an MseI primer without selective nucleotides was combined with a BstYI primer containing either a T or a C as 3' most nucleotide. PCR conditions were as described (Vos et al., 1995). The obtained amplification mixtures were diluted 600-fold and 5 ml was used for selective amplifications using a P33-labeled BstYI primer and the Amplitaq-Gold polymerase (Roche Diagnostics, Brussels, Belgium). Amplification products were separated on 5% polyacrylamide gels using the Sequigel system (Biorad). Dried gels were exposed to Kodak Biomax films as well as scanned in a phospholmager (Amersham Pharmacia Biotech, Little Chalfont, UK).

Characterization of AFLP fragments.

Bands corresponding to differentially expressed transcripts, among which the (partial) transcript corresponding to CDS0689, were isolated from the gel and eluted DNA was reamplified under the same conditions as for selective amplification. Sequence information was obtained either by direct sequencing of the reamplified polymerase chain reaction product with the selective BstYI primer or after cloning the fragments in pGEM-T easy (Promega, Madison, WI) or sequencing of individual clones. The obtained sequences were compared against nucleotide and protein sequences present in the publicly available databases by BLAST sequence alignments (Altschul et al., Nucleic Acids Res. 25 (17) 3389-3402 1997). When available, tag sequences were replaced with longer EST or isolated cDNA sequences to increase the chance of finding significant homology. The physical cDNA clone corresponding to CDS0689 was subsequently amplified from a commercial Tobacco cDNA library as follows.

Cloning of a tobacco CDS0689 seedy1 gene (CDS0689)

A c-DNA library with average inserts of 1,400 bp was made with poly(A+) isolated from actively dividing, non-synchronized BY2 tobacco cells. These library-inserts were cloned in the vector pCMVSPORT6.0, comprising an attB gateway cassette (Life Technologies). From this library 46,000 clones were selected, arrayed in 384-well microtiter plates, and subsequently spotted in duplicate on nylon filters. The arrayed clones were screened by using pools of several hundreds of radioactively labelled tags as probe (among which the BY2-tag corresponding to the sequence CDS0689). Positive clones were isolated (among which the clone reacting with the BY2-tag corresponding to the sequence CDS0689), sequenced, and aligned with the tag sequence. Alternatively, when the hybridization with the tag would fail, the full-length cDNA corresponding to the tag was selected by PCR amplification as follows. Tag-specific primers was designed using primer3 program (http://www-genome.wi.mit.edu/genome_software/other/primer3.html) and used in combination with the common vector primer to amplify partial cDNA inserts. Pools of DNA from 50.000, 100.000, 150.000, and 300.000 cDNA clones were used as templates in the PCR amplifications. Amplification product were isolated from agarose gels, cloned, sequenced and aligned with tags. The vector comprising the sequence CDS0689 and obtained as described above, was referred to as entry clone.

Example 2: Vector construction for transformation with PRO0090-CDS0689 cassette

The entry clone was subsequently used in a Gateway™ LR reaction with p0830, a destination vector used for *Oryza sativa* transformation. This vector contains as functional elements within the T-DNA borders: a plant selectable marker; a plant screenable marker; and a Gateway cassette intended for LR in vivo recombination with the sequence of interest already cloned in the entry clone. The rice prolamin RP6 promoter for endosperm-specific expression (PRO0090) is located upstream of this Gateway cassette.

After the LR recombination step, the resulting expression vector as shown in Fig. 2 was transformed into *Agrobacterium* and subsequently into *Oryza sativa* plants. Transformed rice plants were allowed to grow and then examined for various parameters as described in Example 3.

Example 3: Evaluation of transgenic rice plants transformed with prolamin::seedy1 (PRO0090-CDS0689) and results

Approximately 15 to 20 independent T0 rice transformants were generated. The primary transformants were transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Four events of which the T1 progeny segregated 3:1 for presence/absence of the transgene were retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and approximately 10 T1 seedlings lacking the transgene (nullizygotes), were selected by monitoring screenable marker expression.

Two events (60 plants per event of which 30 positives for the transgene and 30 negative) having improved agronomical parameters in T1 were chosen for re-evaluation in T2. T1 and T2 plants were transferred to the greenhouse and evaluated for vegetative growth parameters and seed parameters, as described below.

Statistical analysis: t-test and F-test

A two factor ANOVA (analysis of variants) was used as statistical model for the overall evaluation of plant phenotypic characteristics. An F-test was carried out on all the parameters measured, for all of the plants of all of the events transformed with the gene of interest. The F-test was carried out to check for an effect of the gene over all the transformation events and to determine the overall effect of the gene or "global gene effect". Significant data, as determined by the value of the F-test, indicates a "gene" effect, meaning that the phenotype observed is caused by more than the presence or position of the gene. In the case of the F-test, the threshold for significance for a global gene effect is set at a 5% probability level.

Vegetative growth measurements

The selected transgenic plants were grown in a greenhouse. Each plant received a unique barcode label to link unambiguously the phenotyping data to the corresponding plant. The selected transgenic plants were grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod= 11.5 h, daylight intensity= 30,000 lux or more, daytime temperature= 28°C or higher, night time temperature= 22°C, relative humidity= 60-70%. Transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. From the stage of sowing until the stage of maturity each plant was passed several times through a digital imaging cabinet and imaged. At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles.

The parameters described below were derived in an automated way from all the digital images of all the plants, using image analysis software.

(a) Aboveground plant area

Plant aboveground area was determined by counting the total number of pixels from aboveground plant parts discriminated from the background. This value was averaged for the pictures taken on the same time point from the different angles and was converted to a physical surface value expressed in square mm by calibration. Experiments show that the aboveground plant area measured this way correlates with the biomass of plant parts above ground.

b) Number of primary panicles

The tallest panicle and all the panicles that overlap with the tallest panicles when aligned vertically were counted manually, and considered as primary panicles.

Seed-related parameter measurements

The mature primary panicles of T1 and T2 plants were harvested, bagged, barcode-labelled and then dried for three days in the oven at 37°C. The panicles were then threshed and all the seeds were collected and counted. The filled husks were separated from the empty ones using an air-blowing device. The empty husks were discarded and the remaining fraction was counted again. The filled husks were weighed on an analytical balance. This procedure resulted in the set of seed-related parameters described below.

(c) Number of filled seeds

The number of filled seeds was determined by counting the number of filled husks that remained after the separation step.

(d) Total seed yield per plant

The total seed yield was measured by weighing all filled husks harvested from a plant.

The results show % difference between positive plants and corresponding nullizygotes (negative) plants of a transgenic line. The values given in Tables 1 to 4 represent the average for two T1 lines and the same two T2 lines.

Table 1: overview of phenotypic data of seedy1 transgenic T1 and T2 plants for above ground area

	% difference between pos. and neg. plants for above ground area		
	T1 plants	T2 plants	
2 lines	+ 51 %		
2 lines		+ 25.5 %	

Table 2: overview of phenotypic data of seedy1 transgenic T1 and T2 plants for number of first panicles

	% difference between pos. and neg. plants for nr. of first panicles		
	T1 plants	T2 plants	
2 lines	+ 101 %		
2 lines		+ 26.5 %	

Table 3: overview of phenotypic data of seedy1 transgenic T1 and T2 plants for number of filled seeds

	% difference between pos. and neg. plants for nr. of filled seeds		
	T1 plants	T2 plants	
2 lines	+ 137 %		
2 lines		+ 36.5 %	

Table 4: overview of phenotypic data of seedy1 transgenic T1 and T2 plants for total seed weight per plant

	% difference between pos. and neg. plants for total seed weight per plant		
	T1 plants	T2 plants	
2 lines	+ 152 %		
2 lines		+ 47 %	